

## Plant growth and symptomatology of macronutrient deficiencies in cowpea plants

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### Abstract

Cowpea is a widely grown annual legume in the North and Northeast regions of Brazil. It is one of the main sources of protein and income for the populations of these regions. However, few studies have shown the nutritional requirements, and symptomatology of nutritional deficiencies of cowpea cultivars. In this context, the objective of this work was to evaluate the initial growth and symptomatology of macronutrient deficiencies in cowpea plants of the BR17-Gurguéia cultivar. A randomized complete experimental design with three replications was used, with seven treatments consisted of nutrient solutions containing no N, P, K, Ca, Mg, or S, and a nutrient solution containing all macro and micronutrients as control, using one plant per plot. Plant height, stem diameter, number of leaves, leaf area, root volume, and shoot and root dry weights were evaluated, and visual symptoms of nutritional deficiencies were described at 30 days after planting. Nitrogen, phosphorus, potassium, calcium, and magnesium were the most limiting nutrients for the vegetative growth and biomass production of cowpea up to 30 days of cultivation. The absence of these elements in the nutrient solution caused morphological changes and visual symptoms that are characteristic of the nutritional deficiency of these nutrients.

**Keywords:** BR17-Gurguéia, nutritional deficiency, mineral nutrition, *Vigna unguiculata* (L.) Walp

### Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is widely cultivated and is an important source of food and income for small and medium farmers in the Northeast region of Brazil. Cowpea is the third most economically important crop in the state of Piauí, with an area of 214,137 ha, producing 98,807 Mg of beans, with average grain yield of 461 kg ha<sup>-1</sup> (IBGE, 2016). Moreover, this crop generates nearly 210,000 jobs in this state, whose production can feed more than 3 million people (Freire Filho et al., 2007). Despite this significant production, the average grain yield of this crop is low, especially due to the low fertility, and lack of adequate fertilization management of the soils in

which it is planted.

Fertilization management is determinant for the nutritional balance of crops, with consequent increase in grain yield and nutritional value. When the plant has a nutritional deficiency, it expresses this imbalance by visual symptoms, which are mainly shown by alterations in its leaves, such as changes in color and size, since most of the physiological and biochemical activities of the plant occurs in them (Ramos et al., 2009). In addition, some symptoms are also shown by fruits since nutritional deficiencies can reduce their yield and commercial value.

BR17-Gurguéia is one the most commonly used cowpea cultivars in the state of Piauí. It

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is well accepted in the Piauí market, mainly because it has characteristics of always-green varieties, which increases its commercial value. In addition, this cowpea cultivar presents high resistance to the cowpea severe mosaic virus (CpSMV) and cucumber mosaic virus (CMV).

Freire Filho et al. (1999) evaluated the BR17-Gurguéia cultivar and found an average grain yield of 976 kg ha<sup>-1</sup>, which was 18%, and 49% higher than those of the BR10-Piauí, and CE-315 cultivars, respectively, when it was grown without irrigation. When it was grown with irrigation, it showed an average grain yield of 1,695 kg ha<sup>-1</sup>, which was 32%, and 60% higher than those of the BR10-Piauí, and CE-315 cultivars, respectively.

Several studies have been carried out using the BR17-Gurguéia cultivar in Piauí. However, no studies showed the nutritional requirements of this cultivar and the symptomatology of macronutrient deficiencies. In this context, the objective of this work was to evaluate the initial growth and symptomatology of macronutrient deficiencies in cowpea plants of the BR17-Gurguéia cultivar.

### Material and methods

The experiment was conducted in a greenhouse with 50% luminosity at the Federal University of Piauí, in Bom Jesus-PI (09°04'28"S, 44°21'31"W, and average altitude of 277 m). The experiment was conducted in a randomized complete experimental design with seven treatments and three replications. The treatments applied to the cowpea (*Vigna unguiculata* (L.) Walp) plants of the BR17-Gurguéia cultivar consisted of nutrient solutions containing no nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), or sulfur (S), and a nutrient solution containing all these macronutrients and micronutrients (Hoagland & Arnon, 1950) as control.

Four seeds were sowed per pot and the seedlings were thinned to one plant per pot, using Leonard pots made from pet bottles. The substrate used was sand, which had been washed with 1% HCl solution and distilled water and sterilized in autoclave at 120 °C for one hour; this procedure was carried out in two consecutive days. Each bottle was filled with 1.5 kg of sand

and 1 L of nutrient solution, which reached the substrate by capillarity.

Plants were kept in a diluted nutrient solution of ¼ of the original concentration during the first five days for acclimatization purposes. The pH of the nutrient solution was monitored daily during the experimental period and adjusted to 6.0±0.5, using either NaOH or HCl 0.1 mol L<sup>-1</sup>. The water lost by evapotranspiration was replenished using distilled water and the original nutrient solution was renewed weekly.

The evolution of the nutritional-deficiency symptoms was monitored through descriptions and photographs. The symptoms were well defined at 30 days after sowing; the heights of the plants from the soil surface to the last trifoliate leaf were then determined using a millimeter-graduated ruler; the stem diameter was measured at 0.02 m from the soil surface using a digital caliper (Digimes®); the number of leaflets, and trifoliate leaves were counted; and foliar area was measured using an automatic leaf area meter (LI-3100C, Li-Cor, Biosciences®).

The roots were separated from the shoots to determine their volume, which was measured by water displacement in a graduated cylinder. The shoots and roots were dried in a forced-air circulation oven at 65 °C for 72 hours to determine their dry weights.

The data were subjected to analysis of variance and the means were compared by the Skott Knott test at 5% probability, using the Sisvar software (Ferreira, 2014).

### Results and discussion

#### Vegetative growth

Plants subjected to nitrogen deficiency showed lower plant height compared to the other treatments, with a 65% reduction when compared to the control (Table 1). Nitrogen acts on ionic absorption, photosynthesis, respiration, and cell multiplication and differentiation, increasing vegetative growth, biomass accumulation, and leaf area (Malavolta, 2006). In addition, N is the most absorbed nutrient by cowpea plants and has the greatest effect on the crop development (Miranda et al., 2017). The positive response of plants to the use of this nutrient has been reported in the literature (Barbosa et al., 2010, Bennett et

al., 2013, Santos et al., 2016).

The number of leaves reduced significantly in plants subjected to N, P, or Mg deficiency compared to the control (Table 1). These nutrients are directly linked to important processes to plant development, such as ATP synthesis, respiration, photosynthesis, chlorophyll formation, enzymatic activation, ionic absorption, and energy transport (Malavolta, 2006). The reduction in number of leaves is also linked to the existing synergistic interaction between these nutrients (Malavolta, 1980), since the absence of one can compromise the efficiency

of the absorption of the other, limiting vegetative growth.

The stem diameter, leaf area, and root volume of plants with N, P, K, Ca, or Mg deficiency reduced when compared to the control (Table 1). A study carried out with common bean crops subjected to macronutrient deficiency showed decreases in leaf area (93%) and stem diameter (32%) of plants due to phosphorus deficiency (Leal & Prado, 2008). Ca is one of the most limiting nutrients to root development (Malavolta, 1980); it decreased the root volume in 75% when compared to the control.

**Table 1.** Plant height (PH), stem diameter (SD), number of leaves (NL), leaf area (LA), root volume (RV), shoot dry weight (SDW), root dry weight (RDW), and total dry weight (TDW) of cowpea plants of the BR17-Gurguéia cultivar grown under deficiency of macronutrients.

Treatments	PH	SD	NL	LA	RV	SDW	RDW	TDW
	----cm----	--mm--	-	---cm <sup>2</sup> ---	---cm <sup>3</sup> ---	-----g plant <sup>-1</sup> -----		
Control	23.66 a	4.29 a	3.0 a	309.00 a	18.33 a	1.76 a	1.03 a	2.79 a
N deficiency	8.20 c	1.70 b	1.0 b	195.00 b	3.66 b	0.14 b	0.12 b	0.26 b
P deficiency	13.50 b	2.77 b	1.3 b	60.33 b	3.66 b	0.27 b	0.18 b	0.45 b
K deficiency	12.23 b	2.66 b	2.6 a	123.33 b	5.00 b	0.42 b	0.33 b	0.75 b
Ca deficiency	14.16 b	3.41 b	3.0 a	117.33 b	4.66 b	0.68 b	0.31 b	0.99 b
Mg deficiency	12.76 b	2.44 b	2.0 b	105.66 b	6.00 b	0.51 b	0.23 b	0.74 b
S deficiency	24.66 a	4.81 a	4.0 a	561.33 a	13.33 a	2.26 a	0.97 a	3.23 a
Mean Square	133.42*	3.18*	3.04*	91.68*	4.19*	0.49*	0.21*	4.03*
CV (%)	23.55	22.20	33.08	35.98	27.44	24.89	35.68	27.37

Means followed by the same letters do not differ by the Scott-Knott test at 5% probability. \* =  $p < 0.05$ ; CV = coefficient of variation.

Reductions in the variables of plants subjected to K deficiency can be explained by the interaction of this nutrient with others; for example, K is involved in the beginning of the metabolic processes of N-incorporation of mineral N, and nitrate reduction.

The absence of any of the macronutrients, except S, decreased the shoot, root, and total dry weights (Table 1). The low biomass production of the plants is probably connected to the growth parameters, since they show the limitations of the initial growth caused by nutrient deficiencies. Studies on plants treated with absence of nutrients in the nutrient solution showed that N and Ca were the most limiting nutrients to biomass production in cowpea (Miranda et al., 2010; 2017).

Plants subjected to S deficiency had similar initial growth to those of the control (Table 1). Probably, this nutrient was supplied through atmospheric deposition. This result was similar those found by Leal & Prado (2008) in common bean plants, with all growth parameters

presenting no effect due to the absence of S in the nutrient solution when compared to the control, except plant height. The results of Leal & Prado (2008) differ from the results of Miranda et al. (2010) in cowpea, who found a reduction of 51% in the dry weight of plants with no S in the nutrient solution.

#### Symptomatology

The initial symptoms of nitrogen deficiency were characterized by the light-yellow green color of mature leaves, which caused a reduction on the development of the plants (Figure 1a). This nutrient is the main constituent of proteins, nucleic acids, phytohormones, and secondary metabolites (Malavolta, 1980), especially the chlorophyll, which is a green-color pigment that is indispensable for the photosynthesis process.

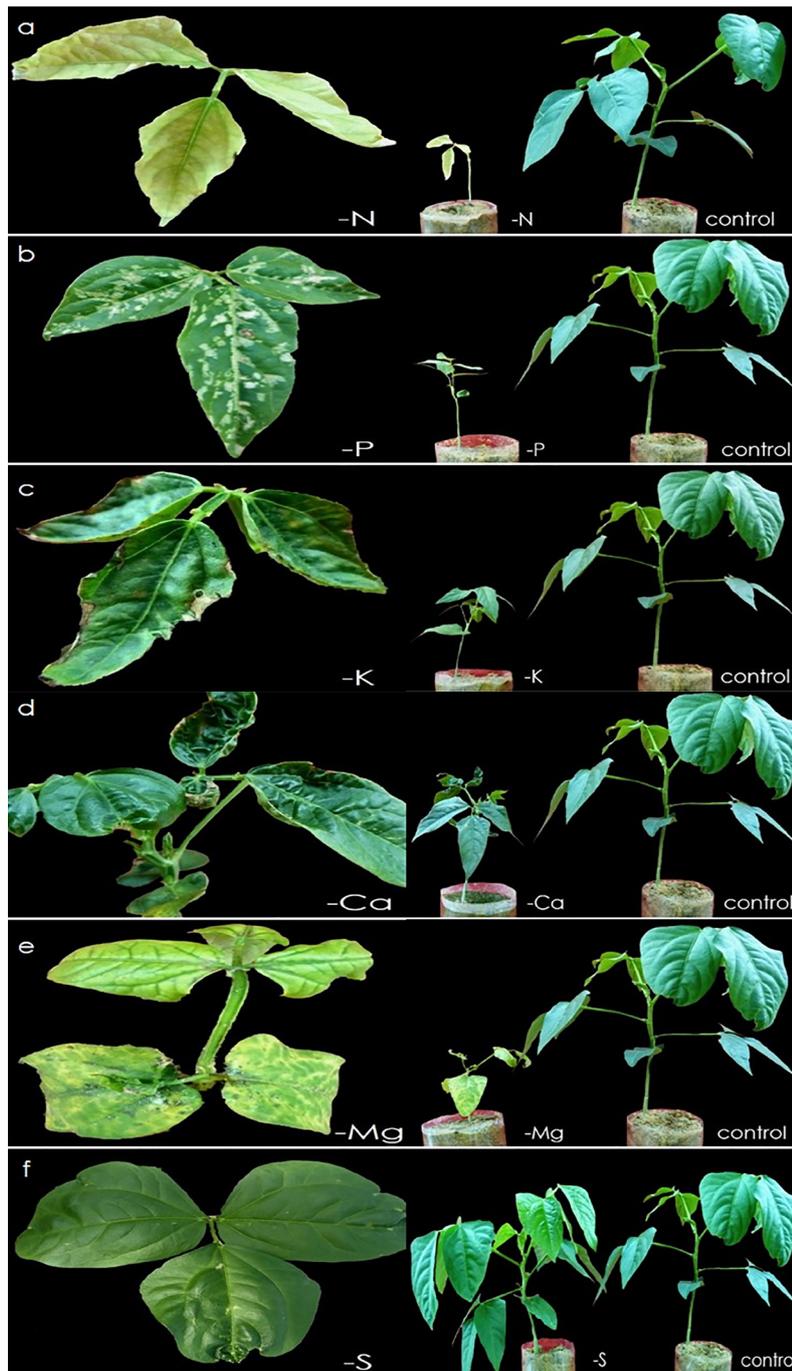
The plants subjected to P deficiency presented visual symptoms such as narrow leaf insertion angle, opaque dark green color, low vegetative development, and leaves with homogeneous distribution of chlorotic points

in the internodal regions followed by necrosis, and upward curved edges (Figure 1b). These symptoms were also observed by Miranda et al. (2010) in cowpea plants, and by Leal & Prado (2008) in common bean plants.

Potassium was the nutrient that caused the third greatest reductions in the vegetative growth of cowpea, after N and Mg. The symptoms of potassium deficiency in the plants consisted of mature leaves becoming opaque green, with

dark punctures in the borders of the leaf limbs, followed by chlorosis, and intense necrosis (Figure 1c).

These symptoms were due to the functions of K in the plant, especially the osmotic potential regulation, and enzymatic activation (Malavolta, 2006). The necrosis in the leaf edges shows the water unbalance of the plant, which is initially seen in the region where most of the stomata are concentrated.



**Figure 1.** Symptoms of nitrogen (a), phosphorus (b), potassium (c), calcium (d), magnesium (e), and sulfur (f) deficiencies in cowpea plants of the BR17-Gurguéia cultivar.

Plants growing in the absence of Ca were the first to show characteristic symptoms-wrinkled and upward curved edges of young leaves, dark punctures throughout the leaf limbs, and chlorosis progressing to necrosis. The leaves appeared to be thick and brittle and presented intense green opaque color (Figure 1d); moreover, they were easily detached from the stem. The same symptoms were observed by Miranda et al. (2010) in cowpea plants. The symptoms of these plants were characteristic of Ca deficiency, due to its main functions in the plant, since it is a nutrient with little mobility, and binds to polysaccharides in the cell wall (Malavolta, 2006); therefore, the symptoms were more pronounced in meristematic regions.

The symptoms of Mg deficiency were poor leaf formation, with irregular borders, and marked internal chlorosis. This chlorosis occurs because Mg is an important constituent of the chlorophyll, a greenish pigment found in chloroplasts that is responsible for photosynthesis. Furthermore, plants in this treatment showed presence of sucking insects that probably have been attracted due to their nutritional imbalance. The hypothesis that explains this result is that the absence of Mg causes a higher K uptake due to the antagonistic interaction between these nutrients; the higher concentrations of K in the plant possibly increased the K to N ratio and consequently increased synthesis of soluble compounds of low molecular weight that favored the attack of pests and diseases to the plants (Seabra Júnior et al., 2013).

The absence of S in the nutrient solution caused no significant effects on vegetative growth parameters and the plants showed no symptoms of deficiency during their initial growth (Figure 1f). However, Miranda et al. (2010) evaluated nutritional deficiencies of macronutrients in cowpea seedlings and observed visual symptoms of sulfur deficiency on the eleventh day of experiment.

### Conclusions

Nitrogen, phosphorus, potassium, calcium, and magnesium were the most limiting nutrients for the vegetative growth and biomass production of cowpea up to 30 days of

cultivation. The absence of these elements in the nutrient solution caused morphological changes and visual symptoms that are characteristic of nutritional deficiency of these nutrients.

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